Annals of Clinical and Analytical Medicine
Original Research

Effect of moderate exercise and vitamin D3 on adipose tissue SIRT1 Protein in obese rats

Hanan Salih Abdulrraziq[1], Hala Mahmoud Ibrahim Abou Heif[2], Hala Mohamed El-Sayed Maklad[2], Gehan Yassin Soliman Shoeib[2], Mona Hassan Fathelbab[3]
[1] Department of Medical Physiology, Faculty of Medicine, Omar AlMukhtar University, Albayda, Libya
[2] Department of Medical Physiology, Faculty of Medicine, Alexandria University, Alexandria, Egypt
[3] Department of Medical Biochemistry, Faculty of Medicine, Alexandria University, Alexandria, Egypt

Abstract
Aims: Obesity is a chronic metabolic disease affecting almost all physiological functions of the body leading to increased morbidity and mortality. The increased prevalence of obesity has been attributed to several risk factors that contribute to several metabolic dysfunctions.
This study was conducted to evaluate the effect of moderate exercise and vitamin D3 on adipose tissue SIRT1 protein level in obese rats.
Material and Methods: The study was carried out on 40 adult male Wister albino rats, divided into Group 1 (control group): 8 rats received a standard rat diet for 12 weeks and Group 2 (obese group): 32 rats received a high fat diet (HFD) for 4 weeks. Group 2 was further subdivided into 4 subgroups each of 8 rats; (a) Control sedentary group (b) Exercise group (c) Vitamin D3 supplement group (d) Exercise plus vitamin D3 supplement group. At the end of 12 weeks, the anthropometric index and adipose tissue SIRT1 protein by ELISA were measured.
Results: HFD results in abnormal anthropometric data and a significant reduction of SIRT1 protein in adipose tissue. In addition to elevation of SIRT1 protein in combined treatment, there was a negative correlation between SIRT1 protein and adiposity index.
Discussion: The combination of VD and exercise improved significantly anthropometric parameters and increased adipose tissue SIRT1 protein.

Keywords
Obesity, VD3, Exercise, SIRT1

DOI: 10.4328/ACAM.22073 Received: 2023-12-16 Accepted: 2024-01-22 Published Online: 2024-01-26 Printed: 2024-03-01 Ann Clin Anal Med 2024;15(3):199-203
Corresponding Author: Hanan Salih Abdulrraziq, Department of Medical Physiology, Faculty of Medicine, Omar AlMukhtar University, Albayda, Libya.
E-mail: hnansaleh83@gmail.com P: +20 101 671 66 43
Corresponding Author ORCID ID: https://orcid.org/0009-0006-2771-6545
This study was approved by the Ethics Committee of Alexandria University (Date: 2022-06-16, No: 0201690)

Introduction

Obesity is the outcome of complex relations between environmental, genetic, and epigenetic factors. The two main factors that are thought to contribute to obesity are excessive consumption of high-energy foods and physical inactivity [1].

One potential tactic for the prevention and/or treatment of obesity and its related complications has been to focus on the development and functional alterations of adipose tissues during the obesity process. Obese people frequently have low vitamin D levels, which are associated with a higher risk of obesity [2]. Sirtuins (SIRT1) is a family of NAD-dependent enzymes that deacetylate different proteins' lysine residues. It seems that adipose tissue is a significant SIRT1 action site. It has been documented that a high-fat diet (HFD) increased insulin resistance by causing the proteolytic cleavage of SIRT1 [3].

Exercise is a crucial non-pharmacological tool that has a profoundly positive impact on the body's many functional systems. Nevertheless, the precise process by which regular exercise improves organ function is still unclear.

As a result, this research was performed to explore their effect on SIRT1 protein in adipose tissue in a rat model of HFD-induced obesity.

Material and Methods

2.1 Experimental design:

The present study involved 40 healthy male Wister albino rats aged 2-3 months with body weights from 150- 180 g each. Animals were housed in separate cages. They were maintained in normal light and temperature conditions with unrestricted access to food and water. Before the study began, the animals were allowed to adjust to their new housing for a week. All institutional and national guidelines for the care and use of laboratory animals were followed.

All the animals were randomly divided into two groups. Group 1, the normal control group (NC), included 8 rats. They served as healthy controls and received a standard rat diet for 12 weeks. Group 2, the high-fat diet group (HFD), included 32 rats and received HFD for 4 weeks. The HFD was used to make rats obese, and it contained 20 g of fat per 100 g of food (To supply essential fatty acids, use 1 g of soybean oil and 19 g of butter oil) [4].

Then Group 2 was subdivided into 4 subgroups; each of 8 rats. Group 2A: sedentary HFD control group (Sed): rats received HFD for another 8 weeks; Group 2B: Exercise group (Exe): rats received HFD and did swimming exercise for 8 weeks; Group2 C: Vitamin D supplement group (VD): rats received HFD and were given oral VD supplementation (10 mcg/kg /day) [5] for 8 weeks purchased from (medical union pharmaceutical, Egypt). In addition, Group 2 D: Exercise plus vitamin D supplement group (combined): rats received HFD, vitamin D (10 mcg/kg /day) for 8 weeks and did swimming exercise for 8 weeks.

2.2 Swimming exercise protocol.

In the exercised group, rats were subjected to free swimming in a pool filled with 32 ± 1°C water. [6] The protocol was conducted 5 days/ week between 9 and 12 a.m. for 8 weeks. Each swimming session lasted for 30 minutes [6].

2.3 Anthropometric measurement

The rats' weights were measured weekly from the beginning of the experiment. At the end of the experiment body length, weight, body mass index(BMI) and Lee index= cube root of final body weight (g)/ length (cm) [7] were measured for all groups. Furthermore, after anesthesia (isoflurane 4% inhalation) visceral white adipose tissues from the epididymal, mesenteric, perirenal, and retroperitoneal regions were dissected, weighed and the adiposity index was calculated (total visceral fat/final body weight) × 100) [8].

2.4 Determination of SIRT1 protein in adipose tissue.

A. Adipose tissue preparation

After removing any remaining blood, visceral adipose tissue was stored at -80 °C after being cleaned with pre-cooling PBS buffer (0.01M, pH=7.4). After homogenizing the frozen tissues in 100 mg tissue/ml cold PBS with a protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO. USA), the samples were centrifuged for 15 minutes at 12000 xg, and aliquots of the supernatant were taken for ELISA analysis to determine the presence of SIRT1 protein [9].

B. Determination of adipose tissue SIRT1 protein by ELISA.

Adipose tissue supernatant was used to determine adipose tissue SIRT1 protein using Rat SIRT1 ELISA Kit (Catalog No. ER1338, Fine Test, Wuhan, China, e-mail: www.fn-test.com) based on sandwich enzyme-linked immune-sorbent assay technology according to the manufacture instructions. The samples were analyzed in duplicate and SIRT1 protein was expressed in ng/ml. In addition the supernatant of adipose tissue homogenate was used for the estimation of total protein by Lowry's method using folin phenol reagent with bovine serum albumin as a standard and the samples were analyzed in duplicate [10].

The results of adipose tissue SIRT1 ELISA was normalized to total tissue proteins and expressed in ng\mg protein.

Statistical analysis of the data

Data were analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). To confirm that the distribution was normal, the Shapiro-Wilk test was performed. Measures like mean and standard deviation were used to describe quantitative data. The results were deemed significant at the 5% level. The Post Hoc test (Tukey), one-way ANOVA test, and Pearson coefficient were utilized.

Ethical approval

This study was approved by Ethics committee of the Alexandria University (Date: 2022-06-16, No: 0201690).

Results

3.1 Anthropometric parameters

A. Comparison between the different studied groups according to body weight in (gm) in week 1, 4 and 12: In week 1 no significant difference between studied groups (P = 0.885) . while In week 4 There was a significant increase in body weight in groups that took HFD in comparison to normal control group (P <0.001). In addition, in week 12 exercise and combined groups showed no significant difference when compared to normal control groups and a significant decrease in body weight when compared to the sedentary HFD group.

B- Comparison between the different studied groups according to (Adipose tissue weight, BMI, lee index and adiposity index):

The mean of adipose tissue weight, BMI, lee index and adiposity index showed a significant difference between studied groups. in addition exercise and combined groups showed no significant difference when compared to normal control groups and a significant decrease in adipose tissue weight, BMI, lee index and adiposity index when compared to sedentary HFD group. (table1)

3.2 Adipose tissue SIRT1 protein.

There was a significant decrease in SIRT1 protein in the sedentary HFD group in comparison to the normal control group, while no significant difference in exercise and VD groups in comparison to normal control group (table 2).

In addition, there was a significant increase in SIRT1 protein in combined group in comparison to normal control group.

Furthermore, there was a significant increase in SIRT1 protein in VD, exercise and combined group in comparison to sedentary HFD group and its level showed no significant difference between VD and exercise groups.

Our study showed that SIRT1 protein had a significant negative correlation with Adiposity index (r=-0.415, p=0.008) in total sample (n =40)(figure1)

Discussion

Animal obesity brought on by a high-fat diet has been documented in a number of studies, and it is regarded as the most widely used and trustworthy model for researching obesity.

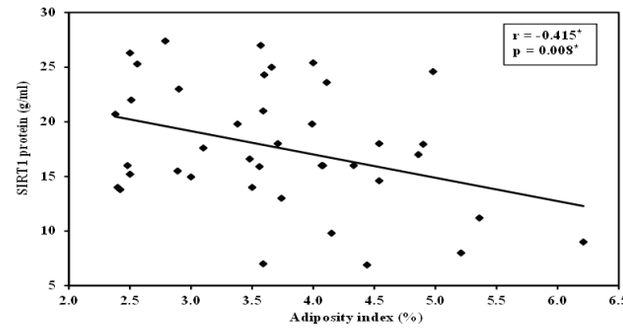

Figure 1. Correlations between SIRT1 protein (ng/mg) and Adiposity index in total sample (n =40)

Table 1. Anthropometric parameters (Adipose tissue weight, BMI, lee index and adiposity index) in different studied groups.

	Normal control	Sedentary HFD control	Exercise	Vitamin-D	combined	F	p
Adipose tissue weight (gm)	7.54c ± 2.51	13.64a ± 3.15	8.85bc ± 2.19	11.10ab ± 2.45	8.73bc ± 1.86	7.699*	<0.001*
p1		<0.001*	0.824	0.049*	0.870		
p2			0.004*	0.262	0.003*		
Sig. bet. grps.			p3=0.377,p4=1.000,p5=0.324				
Length (cm)	18.88a ± 0.35	18.13b ± 0.35	18.75ab ± 0.46	18.50ab ± 0.53	18.75ab ± 0.46	3.694*	0.013*
p1		0.013*	0.979	0.442	0.979		
p2			0.053	0.442	0.053		
Sig. bet. grps.			p3=0.785,p4=1.000,p5=0.785				
BMI (gm/cm2)	0.69c ± 0.05	0.90a ± 0.09	0.74bc ± 0.07	0.81ab ± 0.07	0.73bc ± 0.04	12.068*	<0.001*
p1		<0.001*	0.571	0.011*	0.687		
p2			<0.001*	0.058	<0.001*		
Sig. bet. grps.			p3=0.289,p4=1.000,p5=0.209				
Lee index (g/cm)	0.331c ± 0.010	0.367a ± 0.014	0.340bc±0.013	0.352ab±0.014	0.339bc±0.009	10.513*	<0.001*
p1		<0.001*	0.624	0.017*	0.707		
p2			0.001*	0.100	<0.001*		
Sig. bet. grps.			p3=0.329,p4=1.000,p5=0.264				
Adiposity index (%)	3.05b ± 0.88	4.60a ± 0.91	3.39b ± 0.70	4.02ab ± 0.76	3.39b ± 0.71	4.774*	0.004*
p1		0.004*	0.911	0.133	0.912		
p2			0.035*	0.597	0.035*		
Sig. bet. grps.			p3=0.527,p4=1.000,p5=0.525				

8 replica for each group, Data was expressed using Mean ± SD. SD: Standard deviation, F: F for One way ANOVA test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Tukey), p: p value for comparing between the studied groups, p1: Normal Control in comparison to each other group, p2: Sedentary HFD in comparison to each other group, p3: Exercise in comparison to Vitamin-D, p4: Exercise in comparison to combined, p5: Vitamin-D in comparison to combined *: Statistically significant at p ≤ 0.05, Means with any Common letter (a-c) are not significant (OR Means with totally Different letters (a-c) are significant)

Table 2. SIRT1 protein in adipose tissue (ng/mg protein) in different studied groups.

	Normal control	Sedentary HFD control	Exercise	Vitamin-D	combined	F	p
SIRT1 protein (ng/mg)	17.16b ± 3.46	10.11c ± 3.16	17.88b ± 3.62	19.87ab ± 4.0	23.38a ± 4.24	13.739*	<0.001*
p1		0.005*	0.995	0.596	0.016*		
p2			0.002*	<0.001*	<0.001*		
Sig. bet. grps.			p3=0.820,p4=0.041*,p5=0.343				

8 replica for each group, Data was expressed using Mean ± SD. SD: Standard deviation, F: F for One way ANOVA test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Tukey), p: for comparing between the studied groups, p1: Normal Control in comparison to each other group, p2: Sedentary HFD in comparison to each other group, p3: Exercise in comparison to Vitamin-D, p4: Exercise in comparison to combined, p5: Vitamin-D in comparison to combined *: Statistically significant at p ≤ 0.05, Means with any Common letter (a-b) are not significant (OR Means with totally different letters (a-b) are significant)

In the present study, as expected, a significant increase in anthropometric indices was observed in sedentary HFD control compared to normal control (NC) rats.

In support to our study Madkhali et al proved that, when compared to rats fed a standard diet (SD), rats fed an HFD for 12 weeks had significantly higher body weights, BMIs, Lee indices, and food intake [11]. Furthermore, Yang et al found that the body weight, adiposity index were significantly increased in HFD group [12].

Contradictory to our result Roza et al discovered that, in contrast to age-matched normal diet (ND) rats, the female HFD-treated animals displayed a decrease in body weight during the follow-up (8-week) period, even with higher calorie intake, after the fifth week of life. This result could be due to difference in period of study and animal sex [13].

In the current study moderate swimming exercise was effective in reducing anthropometric indices as compared to the sedentary HFD control group.

Exercise interventions are beneficial for overweight or obese people in terms of lowering body weight, BMI, and body fat percentage, according to a previous meta-analysis of three studies [14]. Lim et al found that anthropometric indices including weight, body fat mass (BFM), BMI, and weight circumference (WC) significantly decreased in older women following a 12-week period of aquatic exercise [15].

In the current study, VD supplementation decreased the anthropometric indices as compared to sedentary HFD control group.

Consistent with our study Verma et al found that VD decreases body weight, various adipose tissues weight, BMI, Lee index, as compared to HFD-treated rats [5].

Sergeev verified that a decrease in the weight of white adipose tissue is correlated with an increase in VD3 intake in a mouse model of induced obesity [16].

A contradictory study by Salehpour et al on 85 women with BMI ≥ 25 kg/m2 showed that VD supplementation (VD3 supplement tablet of 25 µg/d)for 12 weeks did not affect body weight, BMI, and percentage body fat (PBF), this could be due to low dose of VD used in the study [17].

In the current study, the combined group showed a significant reduction in anthropometric indices when compared to the sedentary HFD control group.

Kim et al showed that VD supplementation and exercise attenuated body weight gain, abdominal adiposity, and non-alcohol fatty liver (NAFLD) in rats following induction of obesity [18].

Salarinia et al, demonstrated that in women with type 2 diabetes, exercise and VD supplementation had positive synergistic effects on the cardiometabolic profile. The 12-week intervention with exercise plus VD resulted in significant reductions in body weight, BMI and body fat mass [19].

In the present study, HFD caused a significant reduction in adipose tissue SIRT1 protein level as compared to NC group. In addition SIRT1 showed significant negative correlation with adiposity index.

Consistent to our study, Wei et al discovered that the SIRT1 gene and protein expression are decreased in white adipose tissue (WAT) and brown adipose tissue (BAT) upon the administration of a high-fat non-ketotic diet [20].

Another study investigated whether HFD affects the levels of SIRT1. The results of the quantitative RT-PCR analysis of RNA extracted from WAT revealed that mice given an HFD had two times lower levels of SIRT1 mRNA [3].

In the current study, exercise and VD showed a significant elevation in SIRT1 protein level in AT as compared to sedentary HFD control group.

Liu et al demonstrated that moderate aerobic exercise had positive effects on the liver and kidney functions of diabetic mice. Elevated acetylation of nuclear factor κ (NF-κB) is linked to decreased SIRT1 expression, while SIRT1 expression in the kidney and liver is restored during exercise, which suppresses NF-κB activity [21].

In addition, Vargas et al stated that physical activity can boost oxidative metabolism, mitochondrial biogenesis, and SIRT1 expression in skeletal muscles [22].

VD up-regulate SIRT1 protein expression through up-regulation of adiponectin and consequent activation of activated mitogen phosphokinase (AMPK) [23].

Safarpour et al showed that VD supplementation for 8 weeks increases the serum level of SIRT1 Compared to placebo [24].

In addition, Chang et al suggested that VD supplementation may help to improve the increased intramyocellular fat deposition and related muscle mitochondrial changes that come with obesity, while also increasing the activity of AMPK and SIRT1 [25].

Conclusion

HFD induced obesity in rats resulted in abnormal anthropometric parameters. Furthermore, HFD induced a significant decrease in SIRT1 protein in adipose tissue compared to normal rats. In addition, treatment of HFD by exercise or combined treatment (VD+ exercise) normalized the anthropometric abnormalities in the treated groups versus sedentary HFD. This treatment also increases SIRT1 protein in adipose tissue of rats.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and Human Rights Statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or compareable ethical standards.

Funding: *None*

Conflict of Interest

The authors declare that there is no conflict of interest.

References

1. Pereira-Lancha LO, Campos-Ferraz PL, Lancha AH. Obesity: considerations about etiology, metabolism, and the use of experimental models. Diabetes Metab Syndr Obes. 2012;5:75-87.

2. Vranić L, Mikolašević I, Milić S. Vitamin D deficiency: consequence or cause of obesity? Medicina. 2019;55(9):541.

3. Chalkiadaki A, Guarente L. High-fat diet triggers inflammation-induced cleavage of SIRT1 in adipose tissue to promote metabolic dysfunction. Cell Metab. 2012;16(2):180-8.

4. Zhang XY, Guo CC, Yu YX, Xie L, Chang CQ. Establishment of high-fat diet-induced obesity and insulin resistance model in rats. Beijing Da Xue Xue Bao Yi Xue Ban. 2020;52(3):557-63.

5. Verma A, Goyal A, Kaur R, Kamboj A, Jain UK. Beneficial effect of vitamin d on high-fat diet-induced obesity in wistar rats. Asian J Pharm Clin Res. 2016;3:337-40.

6. Tunca U, Saygin M, Ozmen O, Aslankoc R, Yalcin A. The impact of moderate-intensity swimming exercise on learning and memory in aged rats: The role of Sirtuin-1. Iran J Basic Med Sci. 2021;24(10):1413-20.
7. Novelli EL, Diniz YS, Galhardi CM, Ebaid GM, Rodrigues HG, Mani F, et al. Anthropometrical parameters and markers of obesity in rats. Lab Anim. 2007;41(1):111-9.
8. Leopoldo AS, Lima-Leopoldo AP, Nascimento AF, Luvizotto RA, Sugizaki MM, Campos DH, et al. Classification of different degrees of adiposity in sedentary rats. Braz J Med Biol Res. 2016;49(4):e5028.
9. Zahedi H, Hedayati M. The effect of 14 weeks aerobic exercise on resveratrol supplementation on protein UCP-1, SIRT1, PGC-1a in liver tissue, subcutaneous and visceral fat tissue in male wistar rats. J Sport Biosci. 2018;10(1):39-58.
10. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193(1):265-75.
11. Madkhali HA. Morin attenuates high-fat diet induced-obesity related vascular endothelial dysfunction in Wistar albino rats. Saudi Pharm J. 2020;28(3):300-7.
12. Yang H, Xie J, Wang N, Zhou Q, Lu Y, Qu Z, et al. Effects of Miao sour soup on hyperlipidemia in high-fat diet-induced obese rats via the AMPK signaling pathway. Food Sci Nutr. 2021;9(8):4266-77.
13. Roza NA, Possignolo LF, Palanch AC, Gontijo JA. Effect of long-term high-fat diet intake on peripheral insulin sensibility, blood pressure, and renal function in female rats. Food Nutr Res. 2016;60:28536.
14. Stoner L, Rowlands D, Morrison A, Credeur D, Hamlin M, Gaffney K, et al. Efficacy of exercise intervention for weight loss in overweight and obese adolescents: meta-analysis and implications. Sports Med. 2016;46(11):1737-51.
15. Lim BO, Kang SS, Cho JH, Moon JW. The effect of combined aquatic exercise on body composition, muscular function, static balance and visual analogue scale in female elderly with knee osteoarthritis. Asian J Kinesiol. 2018;20(2):1-13.
16. Sergeev IN. Vitamin D-Cellular Ca(2+) link to obesity and diabetes. J Steroid Biochem Mol Biol. 2016;164:326-30.
17. Salehpour A, Hosseinpanah F, Shidfar F, Vafa M, Razaghi M, Dehghani S, et al. A 12-week double-blind randomized clinical trial of vitamin D_3 supplementation on body fat mass in healthy overweight and obese women. Nutr J. 2012;11:78.
18. Kolieb E, Maher SA, Shalaby MN, Alsuhaibani AM, Alharthi A, Hassan WA, et al. Vitamin D and swimming exercise prevent obesity in rats under a high-fat diet via targeting FATP4 and TLR4 in the liver and adipose tissue. Int J Environ Res Public Health. 2022;19(21):13740.
19. Salarinia M, Azizi M, Tahmasebi W, Khalvandi H. Effect of eight weeks of vitamin D supplementation and water-based exercise on cardiometabolic profile in women with type 2 diabetes. Sci Sport. 2023;38(3):283-92.
20. Wei X, Jia R, Wang G, Hong S, Song L, Sun B, et al. Depot-specific regulation of NAD(+)/SIRTs metabolism identified in adipose tissue of mice in response to high-fat diet feeding or calorie restriction. J Nutr Biochem. 2020;80:108377.
21. Liu HW, Kao HH, Wu CH. Exercise training upregulates SIRT1 to attenuate inflammation and metabolic dysfunction in kidney and liver of diabetic db/db mice. Nutr Metab (Lond). 2019;16:22.
22. Vargas-Ortiz K, Pérez-Vázquez V, Macías-Cervantes MH. Exercise and Sirtuins: A way to mitochondrial health in skeletal muscle. Int J Mol Sci. 2019;20(11):2717.
23. Nemeth Z, Kiss E, Takacs I. A healthy balance of homeostasis by epigenetic regulator SIRT1 may prevent the development of a specific" soil" that supports metabolic disorders and related cancers. Curr Over Dise Health Res. 2022;4:71-111.
24. Safarpour P, Daneshi-Maskooni M, Vafa M, Nourbakhsh M, Janani L, Maddah M, et al. Vitamin D supplementation improves SIRT1, Irisin, and glucose indices in overweight or obese type 2 diabetic patients: a double-blind randomized placebo-controlled clinical trial. BMC Fam Pract. 2020;21(1):26.
25. Chang E, Kim Y. Vitamin D ameliorates fat accumulation with AMPK/SIRT1 activity in C2C12 skeletal muscle cells. Nutrients. 2019;11(11):806.

How to cite this article:
Hanan Salih Abdulrraziq, Hala Mahmoud Ibrahim Abou Heif, Hala Mohamed El-Sayed Maklad, Gehan Yassin Soliman Shoeib, Mona Hassan Fathelbab. *Effect of moderate exercise and vitamin D3 on adipose tissue SIRT1 Protein in obese rats. Ann Clin Anal Med 2024;15(3):199-203*

This study was approved by the Ethics Committee of Alexandria University (Date: 2022-06-16, No: 0201690)